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Polyglutamines Placed into Context

Minireview

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Nine inherited neurodegenerative disorders result from polyglutamine expansions. Two recently published papers on spinocerebellar ataxia type 1, together with studies on spinobulbar muscular atrophy last year, indicate that host protein context is the key arbiter of polyglutamine disease protein toxicity. This insight may represent the most important development in the field since the recognition of nuclear inclusions or the propensity of polyglutamine to aggregate. Indeed, an intimate and inextricable relationship may exist between polyglutamine neurotoxicity and the normal interactions, domains, modifications, and functions of the respective disease proteins.

The spinocerebellar ataxias (SCAs) are a heterogeneous group of inherited neurodegenerative disorders that share the common feature of cerebellar degeneration. SCA1 was the first SCA for which linkage was established and, as it turns out, was also the first SCA gene to be cloned (Orr et al., 1993). Patients with SCA1 harbor CAG repeat expansions of >40 triplets in a novel gene named "*ataxin-1*." Thus, SCA1 became the third recognized "polyglutamine repeat disease," one of nine disorders characterized by expansion of an unstable CAG repeat in the coding region of unrelated genes. (In addition to SCA1, this group includes spinobulbar muscular atrophy [SBMA], Huntington's disease [HD], dentatorubral-pallidoluysian atrophy [DRPLA], SCA 2, 3, 6, 7, and 17.) The mapping, gene discovery, and numerous subsequent studies aimed at understanding the pathogenesis of SCA1 are largely the product of a highly successful collaboration between the laboratories of Harry Orr and Huda Zoghbi. With their publication of two more intriguing papers on SCA1 in recent issues of *Neuron* and *Cell* (Emamian et al., 2003; Chen et al., 2003b), it is clear that the Orr-Zoghbi collaboration remains both vital and synergistic though now nearly two decades old.

Once it was recognized that the polyglutamine diseases share some key features, an approach in this field has been to study each disease individually, with the expectation that an advance in our understanding of any of them might be relevant to our understanding of all of them. One example of the validity of this approach was the independent detection of neuronal intranuclear inclusions (NIs) in SCA3 patients and an HD mouse. This finding has been subsequently extended to almost all of the polyglutamine diseases. The publication of two works on SCA1 implicating ataxin-1 phosphorylation and resulting 14-3-3 stabilization of ataxin-1 as part of the disease pathway raises some important questions: Will these most recent observations be relevant to the other polyglutamine repeat diseases? (and perhaps even to additional neurodegenerative disorders involving improper protein folding/turnover)? Or, is this finding a clue to a pathway that is restricted to SCA1 and thus, perhaps, solely relevant to SCA1 cell-type specificity?

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All Roads Lead to a Critical Serine Phosphorylation

The two reports from the Orr and Zoghbi groups involve a series of experiments that span the gamut from proteomic analysis to cell biology to disease modeling in mice and fruit flies. The initial observation that set the stage for subsequent studies was the detection of a phosphoserine-containing peptide that mapped to amino acid position 774–781 and the determination that phosphorylation was occurring on serine 776 (Emamian et al., 2003). Interestingly, an antibody (PN1168) generated to detect phospho-S776-ataxin-1 preferentially stained nuclear-localized ataxin-1 in the original B05 SCA1 mice. The Orr group then set out to produce transgenic mice that express polyglutamine-expanded ataxin-1 with an alanine substituted for serine at position 776. Despite expressing ataxin-1[82Q]-A776 at levels comparable to those seen in the B05 SCA1 mice and, more importantly, in Purkinje cell nuclei, the A776 mutant ataxin-1 transgenic mice showed minimal behavioral and histopathological abnormalities. The tendency of ataxin-1[82Q]-A776 protein not to form aggregates was evidenced by the lack of nuclear inclusions in the SCA1-82Q-A776 mice and by the detection of insignificant quantities of ataxin-1[82Q]-A776 protein in insoluble fractions generated from transfected CHO cells.

The corollary paper delves into the basis of ataxin-1 phosphorylation and how it mediates pathogenesis (Chen et al., 2003b). This study begins with a rather striking experiment that yields the presence of two immunoprecipitated protein bands in COS1 cells transfected with polyglutamine-expanded ataxin-1 that are absent from COS1 cells transfected with ataxin-1[82Q]-A776. Peptide sequencing of these bands by mass spectrometry indicated that they are different isoforms of the 14-3-3 protein. After showing that coexpression of 14-3-3 with ataxin-1 in HeLa cells actually stabilized ataxin-1 protein with a serine at position 776, the Zoghbi group turned to a fruit fly model of SCA1 to evaluate potential modification. Surprisingly, SCA1-14-3-3 double-transgenic flies generated with a leaky UAS promoter in the absence of a GAL4 driver displayed early lethality, even though singly transgenic SCA1 flies lacking the GAL4 driver are relatively normal, consistent with the stabilization of ataxin-1 protein by 14-3-3. In the final section of the paper, ataxin-1 is shown to be a substrate of Akt kinase, and in vitro binding of ataxin-1 by 14-3-3 is demonstrated to be dependent upon Akt phosphorylation. These results suggest a model of SCA1 neurodegeneration in which Akt phosphorylation of polyglutamine-expanded ataxin-1 leads to its stabilization and accumulation through a direct interaction with 14-3-3.

Akt and 14-3-3: Two Proteins that Come with Well Known Reputations and Relationships

One of the most interesting aspects of the two SCA1 papers is the implication of the Akt kinase and 14-3-3 protein in the pathogenesis of a polyglutamine repeat disease. Akt is a kinase that was discovered about 11 years ago, originally known as protein kinase B. Since its discovery, Akt has emerged as a key node in prosurvival signaling pathways that operate within a wide range of cell types (Brazil et al., 2002). Akt is a well established antiapoptotic signaling molecule, having been shown to phosphorylate the BH3-only protein Bad, the forkhead transcription factor FKHRL1, caspase 9, and IκB family members to counteract their proapoptotic potential (Datta et al., 1999). Akt function has been demonstrated to be relevant to the CNS, as IGF-1-dependent survival of cerebellar granule cell neurons appears to be Akt kinase dependent (Linseman et al., 2002).

The 14-3-3 protein family consists of at least seven highly homologous isoforms, which are highly conserved evolutionarily, with the yeast protein BMH1 displaying an impressive 70% amino acid similarity to the epsilon isoform (Tzivion and Avruch, 2002). The 14-3-3 proteins are among the most abundant proteins in the central nervous system, estimated to account for roughly 1% of all soluble protein in the brain. While a diverse range of functions have been suggested for the 14-3-3 protein family, one of 14-3-3′s most important roles is to serve as a regulatory factor that controls a protein's function by binding to it and restricting it to a certain subcellular compartment. Among the best known examples of this are the interactions of 14-3-3 with the Bad protein and FKHRL1, whereby 14-3-3 binding to the phosphorylated versions of these proteins restricts them to the cytosol. Interestingly, the function of 14-3-3 is linked to Akt kinase in these cases, as Akt phosphorylation of Bad and FKHRL1 is required for 14-3-3 binding.

Tantalizing Leads and Unanswered Questions

The wealth of data presented in support of the Akt-ataxin-1-14-3-3 model put forth by the Orr and Zoghbi groups is a testament to the fact that interdisciplinary approaches combined with cross-species modeling studies are a powerful strategy to tackle complicated problems, such as that of neurodegeneration. Of course, certain questions do remain. One issue is where (subcellularly) is ataxin-1 phosphorylation occurring? Immunostaining of cerebellar sections from SCA1 mice with the phosphoserine-specific antibody preferentially labels ataxin-1 protein localized to the nucleus of Purkinje cells (Emamian et al., 2003). Assuming that limited epitope accessibility in the cell bodies and dendrites is not the explanation, then it would appear that ataxin-1 S776 phosphorylation is somehow linked to its transport into the nucleus. As most models of Akt activation posit that Akt localizes to the plasma membrane upon PI3-K interaction, how is it then that ataxin-1 phosphorylation by Akt could be occurring at the nuclear boundary or in association with nuclear transport factors? A related issue is the basis of Akt activation. If basal conditions in the cerebellum do not permit significant activation of Akt to occur, then the activation of Akt would require that a moderate degree of cellular stress or injury take place in the neurons of the cerebellum prior to Akt activation and subsequent ataxin-1 phosphorylation. If this is the case, then the Akt phosphorylation of ataxin-1 may be a key step in the progression of SCA1, but not in its initiation. Another enigmatic question relates to the detection of ataxin-1 protein on Western blot in SCA1 transgenic mice. While lack of detection of the aggregate-prone ataxin-1 S776 protein seems plausible, why is it that the nonaggregating ataxin-1 A776 protein cannot be detected? Given the power of animal models for testing hypotheses of pathogenesis, reasonable next steps might be to use these model systems to further validate the presented schema. If Akt phosphorylation is required for disease pathogenesis, then crossing SCA1 transgenic mice with Akt knockout mice should ameliorate cerebellar degeneration. It would also be nice to generate SCA1 transgenic fruit flies that express ataxin-1[82Q]-A776 protein to confirm that this version of ataxin-1 is innocuous in flies as it was in mice.

The identification of an ataxin-1 serine residue as a site of Akt phosphorylation and a specific 14-3-3 binding interaction appears on the surface to be an unexpected development in the SCA field. However, at least two other autosomal-dominant SCAs result from mutations that likely affect phosphoregulation. SCA12 has been attributed to a nonpolyglutamine encoding CAG repeat expansion in the promoter of *PPP2R2B*, the gene for the brain-specific regulatory subunit of a protein phosphatase (Holmes et al., 1999). While the mechanism of disease is unclear, it seems likely that a CAG repeat expansion in the promoter would alter the expression level of this phosphatase subunit, perhaps affecting the phosphorylation status of certain key proteins. More recently, missense mutations in the regulatory domain of the γ subunit of protein kinase C were reported to be the cause of a dominant nonepisodic cerebellar ataxia (Chen et al., 2003a). Thus, it appears that one pathway of cerebellar degeneration may involve altered protein phosphorylation.

The Importance of Context

In addition to potentially uncovering an entirely novel ataxin-1 modification and interaction pathway, the Orr and Zoghbi papers reinforce an important concept that is now emerging in the polyglutamine field: Disease protein context is key to elucidating the basis of degeneration. While ataxin-1 function is unknown, the fact remains that the ataxin-1 protein contains an interaction domain that is subject to phosphoregulation, and this aspect of ataxin-1 function is just as important as the polyglutamine expansion for disease pathogenesis. The implications of this observation are profound, as this means that an understanding of the domains and functions determined by disease protein *context* will be critical for solving the mystery of why neurons degenerate in the different polyglutamine disorders (see model in Figure 1).

A striking example of the importance of host protein context is provided by the resolution of a decade-old question regarding the gender specificity observed in SBMA. In contrast to the eight other polyglutamine diseases, SBMA displays X-linked inheritance and affects only males. This sex-limited pattern of inheritance has been difficult to reconcile with the toxic gain-of-function mechanism presumably shared by all polyglutamine diseases. SBMA is caused by a polyglutamine expansion within the androgen receptor (AR), a steroid receptor superfamily member that acts as a ligand-inducible transcription factor (La Spada et al., 1991). Until recently, there were two competing hypotheses to explain gender

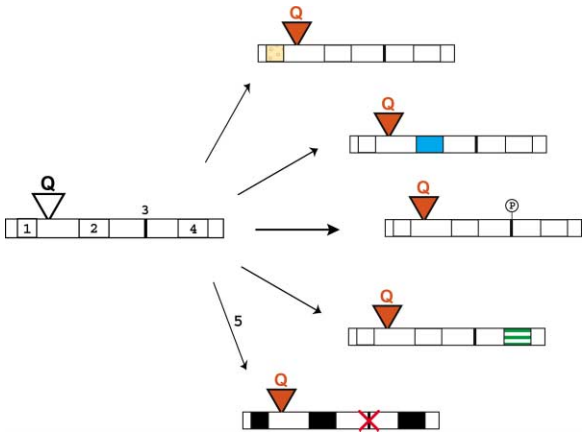

Figure 1. A Model for Polyglutamine Tract Neurotoxicity Based upon the Importance of Host Protein Context

On the left, we see a hypothetical example of a disease protein containing four different domains. The presence of domain 1 may impart toxicity to the polyglutamine tract by augmenting the ability of the expanded glutamine (Q) tract to undergo a conformational change. Domain 2 could be a subcellular localization signal. Domain 3 could be a cleavage site or an amino acid residue subject to phosphorylation. Finally, domain 4 may be an interaction domain that mediates protein binding. While this model emphasizes the role of these domains in imparting pathology to the disease protein, it is also equally possible that the expanded polyglutamine tract eliminates a domain function(s) to cause disease (see #5). The obvious implication of this model is that the normal functions and activities of the host protein are inextricably tied to their mechanism of pathogenesis.

specificity: substantially higher levels of circulating androgens in males versus random (or nonrandom) X inactivation in females. Two recent publications in *Neuron* resolved this issue with surprising clarity. Takeyama et al. expressed full-length AR protein harboring a 52 glutamine stretch in the *Drosophila* eye (Takeyama et al., 2002). Remarkably, the flies exhibited no overt phenotype unless they were exposed to ligand. This ligand-induced degeneration was produced when flies were treated with either agonist or antagonist ligands, demonstrating that translocation to the nucleus is the key event—not induction of gene expression. In an independent study, male transgenic mice expressing full-length AR protein harboring a 97 glutamine stretch showed progressive muscular weakness and atrophy, while female mice showed an insignificant phenotype (Katsuno et al., 2002). Castration of male transgenic mice at puberty prevented degeneration, while administration of androgens to females yielded a pronounced neuromuscular phenotype. In each case, neuromuscular dysfunction correlated with the presence of the polyglutamine-expanded AR in the nucleus. With impeccable timing, a report of the first known cases of two women homozygous for an AR CAG repeat expansion appeared in press two weeks later (Schmidt et al., 2002). Aside from mild cramping, these women exhibited no significant neuromuscular disease or electrophysiological abnormality, ruling out the X inactivation-protection hypothesis. Thus, it is now clear that polyglutamine expansion in the AR protein is not sufficient to produce SBMA.

Primary Target versus Contextual Modulation

The diverse cellular abnormalities observed in models of polyglutamine disease are presumed to reflect a cascade originating from the interaction of the toxic polyglutamine-containing protein with a limited number of primary targets. As it is often assumed that the polyglutamine diseases share a common mechanism, it should follow that primary targets should be shared in the different diseases. This view has been reinforced by a wealth of evidence that expanded polyglutamine, the only feature common to all these diseases, is by itself inherently toxic. Yet, in many cases, the toxicity of expanded polyglutamine, either alone or in the context of truncated host proteins, does not accurately recapitulate key aspects of disease. On the other hand, polyglutamine disease models utilizing full-length protein have been more representative of the phenotype of interest and more useful at dissecting out mechanisms. Such studies have highlighted the importance of host protein context and supported the view that expanded polyglutamine imposes toxic properties on the host protein.

One possible interpretation of this literature is that the toxic gain-of-function resulting from polyglutamine expansion involves an altered or amplified normal function of the host protein and that the primary targets for each disease protein are their normal interacting partners. Such a model of polyglutamine neurotoxicity would account for one of the most intriguing and enigmatic features of these diseases—cell-type specificity. Simply stated, the conundrum is this: while most of the different polyglutamine disease proteins display overlapping patterns of expression throughout the CNS, only certain populations of neurons degenerate in the different diseases. The problem of cell-type specificity is not only relevant to the polyglutamine diseases; indeed, the conundrum exists for other major neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and ALS. One of us has attempted to attack this problem by studying the basis of the cone-rod dystrophy retinal degeneration seen in SCA7. After successfully recapitulating cone-rod dystrophy retinal degeneration in SCA7 transgenic mice, a possible mechanism was uncovered when evidence for a direct physical and functionally significant interaction between ataxin-7 and the cone-rod homeobox protein CRX was found (La Spada et al., 2001). This work led our group to conclude that expansion of a polyglutamine tract in ataxin-7 transformed what was, perhaps, a functional interaction with CRX into a pathogenic encounter. Thus, the basis of one aspect of cell-type specificity in SCA7 might stem from the fact that ataxin-7 normally interacts with CRX.

Subcellular Localization: Uniquely Important, but Not Unifying…

Soon after the recognition of the polyglutamine diseases as a distinct set of disorders, the subcellular site of toxicity became a controversial issue. The debate has traditionally been framed as a choice between the nucleus and the cytoplasm, with the expectation that this determination would provide mechanistic insight. As we now know, most (but not all!) polyglutamine disorders are characterized pathologically by an intranuclear accumulation of mutant protein in the neuronal regions undergoing degeneration. Several disease-causing proteins normally reside in the nucleus, but others that do not (reside in the nucleus) appear to redistribute to the nucleus in disease. Moreover, nuclear localization is required to induce neurodegeneration in disease models (Katsuno et al., 2002; Klement et al., 1998; Saudou et al., 1998; Takeyama et al., 2002). These data have been used

to press the argument that the nucleus is the site where expanded polyglutamine exerts its toxicity. Equally compelling, however, is the apparent absence of nuclear accumulation of the mutant protein in SCA2 and SCA6 (Huynh et al., 2000; Zhuchenko et al., 1997). From the viewpoint of the common polyglutamine toxicity model, this divergence in subcellular localization of aggregates complicates efforts to generate a unifying model. However, if normal functions and normal interactions of the host protein are the key determinants of toxicity, then variation in the subcellular localization of aggregates is not problematic in the least. Indeed, the debate between nucleus and cytoplasm may be too blunt and simplistic a distinction, as different polyglutamine disease proteins appear to be associated with subcellular structures and organelles.

The Role of Context in Determining Disease Threshold

One of the fundamental features of polyglutamine neurotoxicity is the ability of a polyglutamine expansion tract protein to alter its conformation and undergo a structural change that facilitates oligomerization and concurrently causes toxicity (Scherzinger et al., 1997). Studies of polyglutamine aggregation have concluded that a conformation change and its attendant toxicity occur when a threshold of $\sim$35 glutamines is reached. However, the absolute number of repeats necessary to cause disease actually varies with the host protein. In SCA6, a CAG repeat as short as 21 triplets is sufficient to cause disease (Zhuchenko et al., 1997). Comparison of other more orthodox members of the polyglutamine disease group also suggests some differences. For example, onset of disease in SCA3 before age 40 typically requires a repeat expansion of $>$65 CAGs, while onset before age 40 in SCA2 patients usually occurs with $<$45 CAG repeats. Variation in the abilities of the different proteins to accommodate expanding polyglutamine tracts without producing pathology clearly supports the conclusion that host protein context modulates polyglutamine neurotoxicity.

Normal Function as a Guide to Aberrant Function: The "Transcriptionopathy" Hypothesis

Insights into the importance of context in defining the toxicity of expanded polyglutamine suggest that the normal functions and domains of polyglutamine host proteins may serve as a guide to their disease mechanisms. The obvious question then is: what do we know about the normal functions of the other polyglutamine disease proteins? And, might information about their normal function provide clues to the basis of abnormal function? When one surveys the nine known polyglutamine disease proteins, one cannot help but be struck by a recurrent theme: some are well known transcription factors (i.e., AR and TATA binding protein), and a substantial fraction of the remainder (i.e., ataxin-1, ataxin-3, atrophin-1, and huntingtin) have been implicated in transcription regulation. Might, then, the gain-of-function mechanism of polyglutamine expansion protein neurotoxicity involve an alteration of a normal transcriptional function, meaning that many polyglutamine diseases should be considered "transcriptionopathies"?

Closing Thoughts

Although the polyglutamine repeat disease field is barely 12 years old, progress in understanding the basis of polyglutamine disease neurotoxicity has been rapid. While much of the focus has been upon the polyglutamine tract itself, many groups have also devoted themselves to the development of cellular and organismal models. Recent work on SCA1 and on SBMA now suggests that an understanding of the domains, interactions, posttranslational modifications, and functions of the different disease proteins may be critical to uncovering polyglutamine disease mechanisms. We believe that such an approach will be instrumental in moving the polyglutamine disease field forward and ultimately will culminate in the identification of target events and interactions that should be amenable to therapeutic intervention.

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